Conceptus development during blastocyst elongation in lines of pigs selected for increased uterine capacity or ovulation rate

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Conceptus development during blastocyst elongation in lines of pigs selected for increased uterine capacity or ovulation rate[1,2]

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ABSTRACT: Lines of pigs selected for increased uterine capacity have improved conceptus survival, whereas pigs selected for increased ovulation rate have decreased conceptus survival relative to an unselected control line. The objective of this study was to evaluate conceptus development during blastocyst elongation as a potential contributing factor to differences in conceptus survival rate among these pig lines. Conceptuses were recovered from pregnant control, uterine capacity, and ovulation rate line gilts at d 10 and 12 of gestation. At d 10 of gestation, conceptus morphologic diversity was assessed by comparing within-litter average conceptus diameter and the standard deviation of conceptus diameters. At d 12 of gestation, conceptus morphologic diversity was assessed by comparing blastocyst populations obtained from individual gilts. Real-time PCR analyses for transcripts involved in steroidogenesis, cellular differentiation, and immune responsiveness were performed on spherical, ovoid, and filamentous conceptuses recovered from these selection lines. Uterine flushings were also assayed for total protein and estradiol-17β at d 10 and 12 of gestation. Morphological data were analyzed using ANOVA with the fixed effects of line, farrowing season, and their interactions. Conceptus mortality, uterine flushing, and real-time PCR data were analyzed using ANOVA with the fixed effects of line, day or blastocyst morphology, farrowing season, and their interactions. Conceptus mortality, measured as the ratio of conceptus recovery to ovulation rate, was not different between the lines on d 10 and 12 of gestation. There were no significant line effects for conceptus morphologic diversity at d 10 and 12 of gestation. Expression of transcripts associated with steroidogenesis (steroidogenic acute regulatory protein, cytochrome P450 side chain cleavage, and aromatase), cellular differentiation (cytokeratin-18 and vimentin), and immune responsiveness (interleukin-1β) in spherical, ovoid, and filamentous conceptuses was not different between the lines. Furthermore, protein and estradiol-17β in uterine flushings at d 10 and 12 of gestation were not different between the selection lines. These findings indicate limited, if any, deviations between these lines of pigs in conceptus development during blastocyst elongation and suggest that mechanisms involved in generating line differences in survival rate likely are manifested later in gestation.

Key words: blastocyst elongation, estradiol, gene expression, pig, ovulation rate, uterine capacity

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INTRODUCTION

Litter size is an important factor determining the productivity of sows, which has a significant economic impact for the swine industry (Legault, 1985). Two factors that limit litter size include ovulation rate (**OR**) and uterine capacity (**UC**; Bennett and Leymaster, 1989). At the US Meat Animal Research Center (**USMARC**), lines of pigs have been selected for increased OR, increased UC, or randomly (control; CO) over 11 generations of selection (Leymaster and Christenson, 2000). Relative to the CO line, prenatal survival, assessed as a percentage of fetuses or piglets to OR, was increased with selection for UC but decreased with selection for OR (Freking et al., 2007). In a serial slaughter study, analysis of prenatal survival from d 25 to 105

[1]Mention of trade names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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of gestation demonstrated that the greatest deviation in prenatal survival between the UC and OR line occurred between d 25 and 45 of gestation (Freking et al., 2007).

The pig blastocyst undergoes a dramatic morphological transition between d 10 and 12 of gestation, elongating from a spherical to a filamentous morphology, which is essential for proper establishment of pregnancy (Geisert et al., 1982). Although the mechanisms for deviations in prenatal survival between the UC and OR lines are not clear, it is possible that deviations in development of the conceptus (i.e., the embryo and its associated extraembryonic membranes) during blastocyst elongation may reflect differences in survival rates between the selection line pigs. Therefore, the objective of the current study was to test the hypothesis that selection lines differ in conceptus development during blastocyst elongation by comparing 1) conceptus morphologic diversity; 2) transcript expression levels for steroidogenesis, cellular differentiation, and immune responsiveness in conceptuses; and 3) protein and estradiol-17β in the uterine milieu at d 10 and 12 of gestation in these selection lines.

MATERIALS AND METHODS

All animal protocols were approved by the USMARC Animal Care and Use committee and met the USDA guidelines (1995) for the care and use of animals.

Production and Recovery of Blastocysts

Selection for UC was performed using unilateral hysterectomy-ovariectomy of gilts, and selection for OR was performed using laparoscopic examination of gilts over 11 generations in a 4-breed composite having equal contributions of Chester White, Landrace, Large White, and Yorkshire (Leymaster and Christenson, 2000). A randomly selected CO line was maintained using the same 4-breed composite population from which the OR and UC lines were selected. The level of inbreeding was similar between the 3 lines during the selection period (Leymaster and Christenson, 2000). The purebred populations from which the 4-breed composite population was derived were initially sampled in the 1970s (Young et al., 1986). Evaluation of the response to selection for UC and OR has previously been reported (Leymaster and Christenson, 2000; Freking et al., 2007). Each line has been maintained in 40 litters from 10 sires per line in 2 farrowing seasons (March and September) over 8 generations under no intentional selection pressure.

For the current study, normally cycling, intact gilts approximately 200 d of age were checked daily for estrus. At first detection of estrus (designated as gestational d 0), gilts were mated to boars within their respective lines and again 24 h later. A total of 76 gilts from 2 farrowing seasons were randomly assigned to be slaughtered at the USMARC abattoir on either d 10 (n = 13, 13, and 13) or 12 (n = 9, 14, and 14) of gestation from CO, UC, and OR gilts, respectively. Immediately after slaughter, the reproductive tract was removed and processed individually. Total number of corpora lutea (**CL**) was recorded to determine OR. Conceptuses were recovered by flushing each uterine horn of pregnant gilts with 25 mL of ice-cold PBS. Conceptus recovery (**CR**) was determined by counting the number of conceptuses recovered from both uterine horns. At d 12 of gestation, filamentous blastocysts were recovered as clumps, making it difficult to entirely separate individual blastocysts. Therefore, assessment of CR from filamentous clumps was determined by counting individual embryonic discs within each clump using a stereomicroscope. Conceptus mortality within each gilt was determined by the formula [(CL − CR)/CL × 100]. Conceptuses were collected in pools (2 to 5 blastocysts per pool) within individual gilts according to similar morphology (i.e., spherical, ovoid, tubular, and filamentous) and size, snap-frozen in liquid nitrogen, and stored at −80°C until total RNA was extracted. Uterine flushings from each horn were combined, centrifuged at 2,100 × *g* to remove cellular debris, and supernatant was stored at −80°C until analyzed for protein and estradiol-17β content.

Assessment of Morphological Diversity

At d 10 of gestation, the diameters of individual conceptuses were measured, and the standard deviation of conceptus diameter within each litter was determined. These measurements were used to assess conceptus morphologic diversity before blastocyst elongation. Because of the difficulty of entirely separating filamentous clumps and, therefore, accurately measuring filamentous conceptus lengths, the blastocyst population obtained from individual gilts at d 12 of gestation was given a categorical score relative to the combined morphologies of conceptuses recovered (1 = ovoid only; 2 = ovoid and tubular; 3 = ovoid, tubular, and filamentous; 4 = tubular and filamentous; 5 = filamentous only). Within each selection line, categorical blastocyst population scores were averaged to assess conceptus morphologic diversity at d 12 of gestation.

Processing of Total RNA

Spherical (2 mm), ovoid (6 to 8 mm), and filamentous (100 to 150 mm) conceptuses (pooled within gilt) were obtained for each line from individual gilts having uniform conceptus morphology (i.e., spherical conceptuses were selected from gilts with only spherical conceptuses, ovoid conceptuses were selected from gilts with only ovoid conceptuses, and filamentous conceptuses were selected from gilts with only filamentous conceptuses). Total RNA from individual pools of blastocysts was extracted using the RNeasy Mini kit (Qiagen, Valencia, CA) following the protocol of the manufacturer. For removal of genomic DNA, DNase-I treatment was

performed on the spin column using DNase provided by the manufacturer (Qiagen). The concentration of RNA was determined using a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). There were no differences between samples in the quality and integrity of RNA based on the ratio of absorbance at 260 and 280 nm (~2.0) and visualization of 28S and 18S rRNA bands in ethidium bromide-stained agarose gels (data not shown).

Real-Time PCR

Porcine-specific primers (Table 1) utilized to amplify mRNA specific for steroidogenic acute regulatory protein (***STAR***), cytochrome P450 side chain cleavage (***CYP11A1***), aromatase (***CYP19A1***), cytokeratin-18 (***KRT18***), *IL1β*, and ß-actin (***ACTB***) in porcine conceptuses had been previously designed and validated (Ross et al., 2003b; Blomberg et al., 2005, 2006). An additional porcine-specific primer pair was designed using the Primer3 software (Rozen and Skaletsky, 2000) and validated to amplify mRNA specific for vimentin (***VIM***). These transcripts were associated with steroidogenesis (*STAR*, *CYP11A1*, and *CYP19A1*), cellular differentiation (*KRT18* and *VIM*), and immune responsiveness (*IL1B*). A 2-step, real-time PCR method was used for transcript expression analysis in which real-time PCR was performed with the Chromo4 real-time PCR detection system (Bio-Rad, Hercules, CA). Reverse transcription was performed with 500 ng of total RNA isolated from spherical conceptuses (n = 6 pools per line) or 1 μg of total RNA isolated from ovoid (n = 3 pools per line) and filamentous (n = 6 pools per line) conceptuses using the iScript cDNA Synthesis kit (Bio-Rad) according to the protocol of the manufacturer. Each real-time PCR was assayed in duplicate and consisted of 25-ng equivalents of cDNA, 0.25 μ*M* of the appropriate forward and reverse primer, and 12.5 μL of 1× iTaq SYBR Green Supermix with ROX (Bio-Rad) in a 25-μL reaction. All PCR conditions included denaturation (95°C for 2 min) followed by amplification (95°C for 15 s, 60°C for 15 s, and 70°C for 45 s) for 40 cycles. Melt-curve analysis and gel electrophoresis were used to confirm amplification of a single product of the predicted size. The products from a representative sample of PCR were verified by sequence analysis to confirm amplification of the correct cDNA.

Expression levels for each transcript were based on the threshold cycle ($\mathbf{C_T}$) values determined using the Opticon Monitor 3 software (Bio-Rad). For each transcript, 1 assay was performed containing all blastocyst morphologies with an overall average intraassay CV for all transcripts analyzed of 9.4% after converting the exponential C_T to the linear C_T using the formula 2^{-C_T} (Livak and Schmittgen, 2001). Preliminary analysis of the relative expression level (2^{-C_T}) for *ACTB* indicated that *ACTB* did not differ between lines (Table 2) or blastocyst morphologies (Table 3); therefore, *ACTB* was used as the reference control transcript. Calculation of the relative quantity value was determined based on the comparative C_T method ($2^{-\Delta\Delta C_T}$; Livak and Schmittgen, 2001).

Protein and Estradiol-17β Assays

Uterine flushings were measured for protein using the bicinchoninic acid protein assay (Pierce, Rockford, IL) after purification with a trichloroacetic acid precipitation according to the protocol of the manufacturer. All flushings were precipitated, and protein was

Table 1. Porcine-specific primer sequences used for real-time PCR analysis of transcripts in spherical, ovoid, and filamentous conceptuses from pigs selected for increased uterine capacity or ovulation rate relative to a randomly selected control line

Gene ID[1]	Accession no[2]	Primer sequences[3]
STAR	TC252942	F 5′-TGCCGATTTCTCTGCTTCAAC-3′ R 5′-CTGAAAATCTTGGACAGGGATTTT-3′
CYP11A1	TC297588	F 5′-AAGGCCAATGTTACCGAGATG-3′ R 5′-CCAATTGCAGCATCTTGCTTG-3′
CYP19A1	TC238319	F 5′-CATGCGAAAAGCCTTAGAGGA-3′ R 5′-GCTGGAAGTACCTGTAAGGA-3′
KRT18	TC253586	F 5′-GCGAGAAGGAGACCATGCA-3′ R 5′-GGTGTTCCCGGATTTTGATCT-3′
VIM	TC262969	F 5′-AAGGGGACCAACGAGTCTCT-3′ R 5′-TGACATTCAGCAGGTCTTGG-3′
IL1B	TC271146	F 5′-GGCCGCCAAGATATAACTGA-3′ R 5′-CCCTCTGGGTATGGCTTTC-3′
ACTB	TC250404	F 5′-TCCCTGGAGAAGAGCTACGA-3′ R 5′-TAGAGGTCCTTGCGGATGTC-3′

[1]Abbreviations according to human Gene ID (National Center for Biotechnology Information): steroidogenic acute regulatory protein (*STAR*), cytochrome P450 side chain cleavage (*CYP11A1*), aromatase (*CYP19A1*), cytokeratin-18 (*KRT18*), vimentin (*VIM*), ß-actin (*ACTB*).

[2]Accession numbers are from the Dana-Farber Cancer Institute porcine gene index (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb = pig; last accessed 6/20/06).

[3]F = forward primer; R = reverse primer.

Table 2. Line effects for the expression of transcripts associated with steroidogenesis, cellular differentiation, and immune responsiveness in conceptuses recovered from selection line pigs[1]

	Line[2]			
Gene ID[3]	CO	UC	OR	*P*-value
STAR	0.69 (0.59 to 0.80)[4]	0.77 (0.67 to 0.89)	0.72 (0.62 to 0.84)	0.86
CYP11A1	0.40 (0.35 to 0.46)	0.48 (0.42 to 0.54)	0.44 (0.39 to 0.50)	0.64
CYP19A1	0.51 (0.41 to 0.64)	0.79 (0.61 to 0.99)	0.45 (0.36 to 0.60)	0.21
KRT18	1.71 (1.58 to 1.87)	1.48 (1.36 to 1.61)	1.61 (1.48 to 1.75)	0.47
VIM	0.57 (0.46 to 0.71)	0.53 (0.43 to 0.66)	0.46 (0.37 to 0.58)	0.78
IL1B	0.20 (0.16 to 0.24)	0.19 (0.16 to 0.24)	0.22 (0.18 to 0.22)	0.90
ACTB	0.91 (0.82 to 1.02)[5]	0.96 (0.86 to 1.07)	0.87 (0.78 to 0.97)	0.83

[1]Data were log-transformed before analysis and back-transformed to observed values. Values are reported as least squares means. Numbers in parentheses indicate the range of error within 1 SEM after back-transformation.

[2]CO = control; UC = uterine capacity; OR = ovulation rate.

[3]Abbreviations according to human Gene ID (National Center for Biotechnology Information): steroidogenic acute regulatory protein (*STAR*), cytochrome P450 side chain cleavage (*CYP11A1*), aromatase (*CYP19A1*), cytokeratin-18 (*KRT18*), vimentin (*VIM*), ß-actin (*ACTB*).

[4]All target transcript relative quantity based on the comparative threshold cycle (C_T) method ($2^{-\Delta\Delta C}{}_T$).

[5]Reference control transcript expression was measured using the linear C_T value ($2^{-C}{}_T$).

measured in 2 assays with an interassay CV of 3.9% and an intraassay CV of 4.4% (n = 2 per sample). Uterine flushings were also measured for estradiol-17ß as described previously and validated in swine (Redmer and Day, 1981). All flushings were ether-extracted and measured for estradiol-17ß in 1 assay with an intraassay CV of 10.3% (n = 3 per sample).

Statistical Analysis

All data were analyzed using the GLM procedure for ANOVA (SAS Inst. Inc., Cary, NC; Steel et al., 1997), and results are reported as least squares means ± SEM. When a significant F-statistic was determined, means were separated using Tukey-Kramer multiple comparison test (SAS; Steel et al., 1997). The model used for the analysis of morphological data included the fixed effects of line, farrowing season, and their interactions. The model used for the analysis of number of CL, conceptus recovery, conceptus mortality, uterine protein, and uterine estradiol-17ß included the fixed effects of line, day, farrowing season, and their interactions. The model used for the analysis of transcript expression levels included the fixed effects of line, blastocyst morphology (i.e., spherical, ovoid, or filamentous), season, and their interactions. All transcript expression, uterine protein, and uterine estradiol-17ß data were log-transformed before analysis to normalize the data and then back-transformed for reporting observable values.

RESULTS

Conceptus Recovery and Mortality

There were no significant line × gestational day interactions detected for the number of CL ($P = 0.47$), conceptus recovery ($P = 0.12$), or conceptus mortality ($P = 0.48$). Table 4 summarizes the line effects observed for number of CL, conceptus recovery, and conceptus mortality in selection line gilts at d 10 and 12 of gestation. As expected, the total number of CL was greatest in the OR line compared with the CO and UC lines (Table 4). In addition, a significantly greater number of

Table 3. Blastocyst morphology effects for the expression of transcripts associated with steroidogenesis, cellular differentiation, and immune responsiveness in conceptuses recovered from selection line pigs[1]

Gene ID[2]	Spherical (2 mm)	Ovoid (6 to 8 mm)	Filamentous (<100 mm)	*P*-value
STAR	0.008 (0.007 to 0.009)[a,3]	0.05 (0.04 to 0.06)[b]	0.97 (0.85 to 1.11)[c]	<0.001
CYP11A1	0.23 (0.26 to 0.32)[a]	0.29 (0.25 to 0.34)[a]	1.00 (0.89 to 1.13)[b]	<0.001
CYP19A1	0.0006 (0.0005 to 0.0007)[a]	0.29 (0.22 to 0.38)[b]	0.95 (0.77 to 1.17)[c]	<0.001
KRT18	1.92 (1.78 to 2.05)[a]	2.32 (2.10 to 2.58)[a]	0.92 (0.85 to 0.99)[c]	<0.001
VIM	0.37 (0.31 to 0.44)[a]	0.50 (0.38 to 0.65)[ab]	0.77 (0.63 to 0.93)[b]	0.04
IL1B	0.021 (0.017 to 0.026)[a]	0.44 (0.34 to 0.55)[b]	0.92 (0.77 to 1.10)[c]	<0.001
ACTB	0.98 (0.89 to 1.07)[4]	0.80 (0.70 to 0.91)	0.99 (0.89 to 1.09)	0.36

[a–c]Means in the same row with no common superscript differ significantly.

[1]Data were log-transformed before analysis and back-transformed to observed values. Values are reported as least squares means. Numbers in parentheses indicate the range of error within 1 SEM after back-transformation.

[2]Abbreviations according to human Gene ID (National Center for Biotechnology Information): steroidogenic acute regulatory protein (*STAR*), cytochrome P450 side chain cleavage (*CYP11A1*), aromatase (*CYP19A1*), cytokeratin-18 (*KRT18*), vimentin (*VIM*), ß-actin (*ACTB*).

[3]All target transcript relative quantity based on the comparative threshold cycle (C_T) method ($2^{-\Delta\Delta C}{}_T$).

[4]Reference control transcript expression was measured using the linear C_T value ($2^{-C}{}_T$).

Table 4. Line effects for the total number of corpora lutea, conceptus recovery, and conceptus mortality in selection line gilts at d 10 and 12 of gestation[1]

	Line[2]			
Variable	CO	UC	OR	*P*-value
Corpora lutea, n	14.0 ± 0.5[a]	13.7 ± 0.5[a]	18.1 ± 0.5[b]	<0.001
Conceptus recovery, n	11.2 ± 0.7[a]	10.8 ± 0.6[a]	14.0 ± 0.6[b]	<0.001
Conceptus mortality,[3] %	19.9 ± 4.0	21.0 ± 3.5	22.5 ± 3.6	0.89

[a,b]Means in the same row with no common superscript differ significantly.
[1]Least squares means ± SEM.
[2]CO = control; UC = uterine capacity; OR = ovulation rate.
[3]Based on the formula [(CL − CR)/CL × 100].

conceptuses were recovered from the OR line compared with the CO and UC lines (Table 4). However, the recovery rate of conceptuses was not different between the selection lines, and as a result, conceptus mortality was not different between the selection lines (Table 4). There were no significant gestational day effects for the number of CL when combining gilts from the selection lines (Table 5). However, conceptus recovery was significantly decreased at d 12 of gestation compared with d 10 of gestation (Table 5). As a result, conceptus mortality was greater at d 12 of gestation compared with d 10 of gestation (Table 5).

Conceptus Morphological Diversity

The morphologies of conceptuses recovered at d 10 and 12 of gestation ranged from spherical (<4 mm) and ovoid (5 to 8 mm) at d 10 of gestation to ovoid (5 to 10 mm), tubular (11 to 80 mm), and filamentous (>100 mm) at d 12 of gestation. At d 10 of gestation, the mean conceptus diameter was not different between the selection lines (Table 6). Similarly, the standard deviation of conceptus diameter within individual gilts at d 10 of gestation was not significantly different between the lines (Table 6). Furthermore, the mean categorical blastocyst population score obtained at d 12 of gestation was not different between the selection lines (Table 6). These results demonstrate limited deviations in conceptus morphological diversity between the selection lines during early gestation.

Transcript Expression Levels

No significant line × blastocyst morphology interactions were detected for the expression of *STAR* ($P = 0.37$), *CYP11A1* ($P = 0.89$), *CYP19A1* ($P = 0.95$), *KRT18* ($P = 0.66$), *VIM* ($P = 0.56$), or *IL1B* ($P = 0.92$). There were no significant line effects for the expression level of any of the selected transcripts analyzed (Table 2), indicating limited deviations in gene regulation by mRNA abundance for these various cellular processes in conceptuses from gilts selected for increased UC or OR. There were different patterns of expression for the transcripts observed between the 3 blastocyst morphologies analyzed (Table 3). The expression of *STAR*, *CYP11A1*, and *CYP19A1* was significantly increased in filamentous conceptuses compared with spherical and ovoid conceptuses. In addition, the expression of *STAR* and *CYP19A1* was significantly greater in ovoid conceptuses compared with spherical conceptuses. In contrast, *KRT18* expression was significantly decreased in filamentous conceptuses compared with spherical and ovoid conceptuses. The expression of *VIM* was significantly greater in filamentous conceptuses compared with spherical conceptuses, with ovoid conceptuses displaying an intermediate level of *VIM* expression. Furthermore, the expression of *IL1B* was significantly increased in filamentous conceptuses compared with spherical and ovoid conceptuses. In addition, ovoid conceptuses displayed a significantly greater level of *IL1B* compared with spherical conceptuses.

Table 5. Gestational day effects for the total number of corpora lutea, conceptus recovery, and conceptus mortality in selection line gilts at d 10 or 12 of gestation[1]

	Day		
Variable	10	12	*P*-value
Corpora lutea, n	15.5 ± 0.4	15.1 ± 0.4	0.40
Conceptus recovery, n	13.4 ± 0.5	10.6 ± 0.5	<0.001
Conceptus mortality,[2] %	13.6 ± 3.0	28.7 ± 3.1	<0.001

[1]Least squares means ± SEM.
[2]Based on the formula [(CL − CR)/CL × 100].

Table 6. Line effects for conceptus morphologic diversity recovered from selection line gilts at d 10 and 12 of gestation[1]

	Line[2]			
Variable	CO	UC	OR	*P*-value
Blastocyst diameter,[3] mm	2.3 ± 0.4	2.6 ± 0.4	3.1 ± 0.4	0.34
Blastocyst diameter σ,[3] mm	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	0.24
Blastocyst population score[4]	4.0 ± 0.5	4.1 ± 0.4	4.3 ± 0.4	0.87

[1]Least squares means ± SEM.
[2]CO = control; UC = uterine capacity; OR = ovulation rate.
[3]Measurements taken at d 10 of gestation.
[4]Measurements taken at d 12 of gestation. Categorical score: 1 = ovoid only; 2 = ovoid and tubular; 3 = ovoid, tubular, and filamentous; 4 = tubular and filamentous; 5 = filamentous only.

Protein and Estradiol-17β in Uterine Flushings

No significant line × gestational day interactions were detected for protein concentration ($P = 0.33$), total protein ($P = 0.36$), estradiol-17β concentration ($P = 0.47$), or total estradiol-17β ($P = 0.49$). There were no significant differences for protein concentration or total protein in uterine flushings from gilts selected for increased UC or OR compared with CO (Table 7). Similarly, estradiol-17β concentration and total estradiol-17β were not significantly different in uterine flushings from the selection lines. However, there were significant gestational day effects for protein and estradiol-17β in uterine flushings between d 10 and 12 of gestation (Table 8). Protein concentration, total protein, estradiol-17β concentration, and total estradiol-17β were significantly increased in uterine flushings at d 12 of gestation compared with d 10 of gestation.

DISCUSSION

Selection for increased UC improved prenatal survival, whereas selection for increased OR decreased prenatal survival compared with a randomly selected CO line (Leymaster and Christenson, 2000). A serial slaughter study demonstrated that changes in prenatal survival rates between the UC and OR line were greatest between d 25 and 45 of gestation (Freking et al., 2007). Comparison of conceptus development and survival between these lines before d 25 of gestation had not been evaluated previously. Between d 10 and 12 of gestation, the pig blastocyst undergoes a dramatic morphological transition elongating from a spherical structure to a long, thin filamentous structure (Geisert et al., 1982). During blastocyst elongation, estrogen production and secretion by the conceptus increases, serving not only as the signal for maternal recognition of pregnancy (Bazer and Thatcher, 1977) but also as a stimulus for the production of proteins and growth factors within the uterine environment that initiate implantation (Geisert and Yelich, 1997; Geisert et al., 2006). Furthermore, adequate conceptus elongation has downstream effects on pregnancy ultimately affecting conceptus spacing, placental development, and fetal growth, which have implications on UC, litter size, and even postnatal piglet health (Dziuk, 1985). The current study directly compared conceptus development during blastocyst elongation as a potential contributing factor to differences in prenatal survival rates among the CO, UC, and OR lines.

Early embryonic loss, before d 30 of gestation, is a limiting factor to litter size and generally ranges between 20 and 40% (Pope, 1994; Vallet, 2000). In the present study, conceptus mortality was evaluated at d 10 and 12 of gestation from CO, UC, and OR line gilts. At both time points, there was no line effect for conceptus mortality, indicating that differences in conceptus survival rates between these lines are not apparent during blastocyst elongation. However, an increase in conceptus mortality was observed between d 10 and 12 of gestation (13.6 vs. 28.7%, respectively) regardless

Table 7. Line effects for protein and estradiol-17β (E_2) in uterine flushings from selection line gilts at d 10 and 12 of gestation[1]

	Line[2]			
Variable	CO	UC	OR	*P*-value
Protein concentration, mg/mL	0.45 (0.42 to 0.47)	0.45 (0.42 to 0.48)	0.47 (0.44 to 0.49)	0.83
Total protein, mg	19.2 (18.1 to 20.5)	19.0 (18.0 to 20.1)	20.4 (19.3 to 21.7)	0.62
E_2 concentration, pg/mL	66.3 (57.1 to 77.1)	65.1 (57.1 to 74.1)	80.5 (70.5 to 92.1)	0.47
Total E_2, ng	2.9 (2.5 to 3.3)	2.7 (2.4 to 3.1)	3.5 (3.1 to 4.0)	0.39

[1]Data were log-transformed before analysis and back-transformed to observed values. Values are reported as least squares means. Numbers in parentheses indicate the range of error within 1 SEM after back-transformation.
[2]CO = control; UC = uterine capacity; OR = ovulation rate.

Table 8. Gestational day effects for protein and estradiol-17β (E_2) in uterine flushings from selection line gilts at d 10 or 12 of gestation[1]

	Day		
Variable	10	12	*P*-value
Protein concentration, mg/mL	0.31 (0.29 to 0.32)	0.67 (0.64 to 0.70)	<0.001
Total protein, mg	13.3 (12.6 to 13.9)	28.8 (27.4 to 30.2)	<0.001
E_2 concentration, pg/mL	28.5 (25.5 to 31.8)	173.8 (154.9 to 194.9)	<0.001
Total E_2, ng	1.2 (1.1 to 1.4)	7.5 (6.6 to 8.4)	<0.001

[1]Data were log-transformed before analysis and back-transformed to observed values. Values are reported as least squares means. Numbers in parentheses indicate the range of error within 1 SEM after back-transformation.

of selection line. The mortality rates observed in the current study are similar to previously reported mortality for these time points (Pope, 1994). Furthermore, the differences in mortality rate between d 10 and 12 of gestation support the general consensus that the greatest amount of early embryonic loss occurs predominantly between d 12 and 18 of gestation (Pope, 1994; Ashworth et al., 1997).

Several factors have been associated with embryonic mortality including improper timing of conceptus and endometrial development, conceptus signaling failure, embryo-embryo competition, and genetic factors (Geisert and Schmitt, 2002). Diversity among littermate embryos has been shown to play a role in embryonic (Pope et al., 1986; Bazer et al., 1988) and fetal (Pope et al., 1982) mortality. Asynchronous follicle development may result in different patterns of ovulation, thereby resulting in greater diversity among littermate embryos (Pope et al., 1990). It seemed possible that OR line gilts might have greater asynchrony in follicle development due to greater numbers of ova, thereby establishing greater diversity among littermate embryos, which may account for differences in conceptus survival rate seen for the OR line. To test this hypothesis, conceptus morphologic diversity was assessed at d 10 and 12 of gestation from CO, UC, and OR line gilts. At d 10 of gestation, no significant differences between the selection lines were detected for conceptus diameter or the standard deviation of conceptus diameter. Furthermore, blastocyst morphologies obtained at d 12 of gestation did not differ between the selection lines. These results demonstrate that the selection of gilts for increased OR does not affect blastocyst diversity of littermates during the elongation period.

Elongation of the pig blastocyst is not only characterized by a dramatic morphological transition but also represents significant differential gene regulation as the blastocyst transforms from ovoid to filamentous morphology (Ross et al., 2003a; Blomberg et al., 2005). In conjunction with the increase of estrogen production and secretion by the conceptus during blastocyst elongation (Geisert et al., 1990), several transcripts involved in steroidogenesis are increased in a similar pattern as the conceptus elongates from an ovoid to a filamentous morphology (Yelich et al., 1997; Blomberg et al., 2005). For instance, mRNA expression of *STAR*, a protein that mediates transport of cholesterol within the mitochondria, has been shown to increase significantly in filamentous conceptuses compared with ovoid conceptuses (Blomberg et al., 2005). Similarly, mRNA for several enzymes involved in steroidogenesis (i.e., *CYP11A1* and *CYP19A1*) have been shown to be more abundant in the filamentous conceptuses compared with the ovoid conceptuses (Blomberg et al., 2005). In the present study, the greatest expression level of *STAR*, *CYP11A1*, and *CYP19A1* was observed in the filamentous conceptuses compared with the spherical and ovoid conceptuses, which supports previously reported expression patterns for these transcripts (Blomberg et al., 2005). However, there were no significant line effects detected for the expression of *STAR*, *CYP11A*, and *CYP19A1*. Similar expression patterns of steroidogenic transcripts are consistent with results from this study indicating that estrogen production in conceptuses was similar between the selection lines.

Rapid elongation of the pig blastocyst has been primarily associated with cellular remodeling and differentiation rather than cellular hyperplasia as has been described for the elongation of other domestic animal blastocysts (Bazer et al., 1993; Geisert and Yelich, 1997). Potential markers of differentiation of the trophectoderm and mesoderm have been identified in the elongating pig blastocyst (Prelle et al., 2001; Flechon et al., 2004; Blomberg et al., 2006). Cytokeratin-18, an intermediate filament protein, has been shown to play a role in initial establishment of mouse trophoblastic epithelium (Hesse et al., 2000). In the pig, expression of KRT18 protein is localized to the trophectoderm, and expression of *KRT18* mRNA and production of KRT18 protein are decreased in the filamentous conceptus compared with the ovoid conceptus, suggesting that KRT18 plays a role in pig trophectoderm development (Blomberg et al., 2006). Vimentin, another intermediate filament protein localized to mesoderm of the elongating pig blastocyst, has been implicated in mesodermal differentiation and migration (Prelle et al., 2001; Flechon et al., 2004). In the current study, the expression level of *KRT18* was significantly decreased in filamentous conceptuses compared with ovoid and spherical conceptuses, which is consistent with previously reported expression patterns for *KRT18* (Blomberg et al., 2006). The expression of *VIM* was greater in filamentous con-

ceptuses compared with spherical conceptuses, whereas ovoid conceptuses displayed an intermediate level of *VIM* expression. The mRNA expression patterns for *VIM* observed in the current study are consistent with previously observed protein localization patterns of VIM and indicate that greater mesodermal differentiation and migration occur during the filamentous stage (Prelle et al., 2001; Flechon et al., 2004). However, no effect of selection line was detected for the expression of *KRT18* or *VIM*, suggesting limited deviations in trophectoderm and mesodermal differentiation were observed between the selection lines during blastocyst elongation.

During pig blastocyst elongation, the uterine endometrium undergoes a transition to a receptive state that supports conceptus development and subsequent implantation (Burghardt et al., 1997). Proper interactions between the conceptus and uterine environment are essential for establishing the appropriate changes within the uterine endometrium (Geisert et al., 2006). Interleukin-1ß, a proinflammatory cytokine, has recently been implicated in initiating conceptus-uterine communication during pig blastocyst elongation and may play a role in mediating changes within the uterine endometrium (Ross et al., 2003b; Geisert et al., 2006). The present study demonstrated that expression of *IL1B* was greater in filamentous conceptuses compared with spherical and ovoid conceptuses, which is similar to that previously reported (Ross et al., 2003b). However, there was no significant selection line effect for the expression of *IL1B*, indicating that limited deviations in IL1B signaling from the conceptus to the uterine endometrium were observed between the selection lines during blastocyst elongation.

Significant changes in the composition of the uterine milieu have been described during elongation of the pig blastocyst (Geisert et al., 1982, 1990). Protein has been shown to increase in uterine flushings coincident with conceptus elongation (Geisert et al., 1982, 1990). Although the differences in protein in the uterine flushings of cyclic and pregnant gilts are not apparent during the preimplantation period (Vallet et al., 1996, 1998), uterine protein can be used as a general measure of uterine function during blastocyst elongation. Estradiol-17ß also increases in the uterine milieu, with the greatest amounts detected when filamentous stage blastocysts are present (Geisert et al., 1982). The increase in estradiol-17ß measured in the uterine flushing during blastocyst elongation is derived from the elongating conceptus (Heap et al., 1979; Geisert et al., 1982). In the current study, protein and estradiol-17ß were measured in uterine flushings obtained from CO, UC, and OR line gilts at d 10 and 12 of gestation. There was a significant increase of protein and estradiol-17ß (both total amounts and concentrations) in uterine flushings, regardless of selection line, on d 12 of gestation compared with d 10, which supports previously reported patterns of protein and estradiol-17ß (Geisert et al., 1982; Vallet et al., 1998). However, no significant selection line effect was detected for either protein or estradiol-17ß in the uterine flushings. These data indicate limited differences in estrogen production by conceptuses between selection lines, similar to that observed for steroidogenic transcripts. These data further demonstrate limited differences in uterine-conceptus function between the selection lines during blastocyst elongation.

In summary, the results of this study indicate that limited deviations occur in conceptus development during elongation in gilts selected for increased UC or OR, as measured by conceptus morphologic diversity, gene regulation of steroidogenesis, cellular differentiation and immune responsiveness in conceptuses, and protein and estradiol-17ß in the uterine milieu. Furthermore, conceptus survival rates among these selection lines were not different during blastocyst elongation, supporting the previous finding that the greatest changes in conceptus survival rates from these lines occur between d 25 and 45 of gestation (Freking et al., 2007). As a result, the mechanisms involved in generating line differences in conceptus survival rates likely occur at a later time point than d 12 of gestation. Follow-up investigations are ongoing to identify potential mechanisms involved in generation of line differences in survival rates, particularly mechanisms associated with placental development during the critical time in which line differences in conceptus survival become apparent.

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